6. ANALYTICAL METHODS

The purpose of this chapter is to describe the analytical methods that are available for detecting, and/or measuring, and/or monitoring hexachlorocyclohexane, its metabolites, and other biomarkers of exposure and effect to hexachlorocyclohexane. The intent is not to provide an exhaustive list of analytical methods. Rather, the intention is to identify well-established methods that are used as the standard methods of analysis. Many of the analytical methods used for environmental samples are the methods approved by federal agencies and organizations such as EPA and the National Institute for Occupational Safety and Health (NIOSH). Other methods presented in this chapter are those that are approved by groups such as the Association of Official Analytical Chemists (AOAC) and the American Public Health Association (APHA). Additionally, analytical methods are included that modify previously used methods to obtain lower detection limits, and/or to improve accuracy and precision.

6.1 BIOLOGICAL SAMPLES

The α-, β-, γ-, and δ-isomers of HCH, and/or their phenolic metabolites have been measured in biological samples such as adipose tissue, serum, urine, milk, semen, and the brain by gas chromatographic methods listed in Table 6-1.

The most commonly used methods for measuring α-, β-, γ-, and δ-HCH in serum, semen, adipose tissue, and milk are gas chromatography (GC) or high-resolution gas chromatography (HRGC) combined with electron capture detection (ECD) and mass spectrometry (GC/MS) (Barquet et al. 1981; Burse et al. 1990; Butte and Fooken 1990; EPA 1980c; Gupta et al. 1978; LeBel and Williams 1986; Liao et al. 1988; Prapamontol and Stevenson 1991; Saady and Poklis 1990; Stachel et al. 1989; Waliszewski and Szymczynski 1983; Williams et al. 1988). The EPA GC/ECD method is capable of detecting γ-HCH and other HCH isomers in blood serum at the ppb level (EPA 1980c). Using HRGC, method detection limits for measuring HCH isomers in serum and milk are in the sub-ppm to low-ppb range (Butte and Fooken 1990; Prapamontol and Stevenson 1991; Saady and Poklis 1990); recovery and precision are acceptable (Butte and Fooken 1990; Prapamontol and Stevenson 1991; Saady and Poklis 1990). The use of capillary (high-resolution) GC enhances chromatographic separation of compounds with similar retention characteristics (Saady and Poklis 1990). Although GC has also been used in measuring the isomers in blood serum, recovery problems (i.e., low recoveries) have been encountered because of the volatility of the HCH isomers (Burse et al. 1990); sensitivity and precision data were not reported (Burse et al. 1990). GC/ECD combined with identification

Table 6-1. Analytical Methods for Determining Hexachlorocyclohexane in Biological Materials

Sample matrix	Preparation method	Analytical method	Isomer	Sample detection limit	Percent recovery	Reference
Urine	Hydrolyze sample; acidify; extract with hexane; derivatize for GC/ECD or evaporate to a small volume for TLC.	GC/ECD, TLC	Phenolic metabolites of γ-HCH	1 ppb (GC/ECD); 1 ppm (TLC)	95% NR	Balikova et al. 1988
Urine	Hydrolyze acidified sample; extract with diethyl ether; concentrate phenol conjugates	GC/ECD		4.9–18.6 ppb	87–119%	Angerer et al. 1981
Serum	Extract and concentrate serum using solid-phase extraction; elute with isooctane; inject	HRGC/ECD	α-HCH γ-HCH	0.18 ppm 0.33 ppm	70–75%	Saady and Poklis 1990
Serum	Extract serum with organic solvents; sample and acid cleanup on Florisil column; sample cleanup using silica gel chromatography	GC/ECD	β-HCH γ-HCH	NR NR	57.2–58.2% 47.7–50.4%	Burse et al. 1990
Serum	Extract with hexane	GC/ECD	α-HCH β-HCH γ-HCH	1 ppb 1 ppb 1 ppb	NR NR NR	EPA 1980a
Serum	Separate plasma from blood containing anticoagulant	GC/ECD	β-HCH	0.8 ppb	85%	Barquet et al. 1981
Serum	Hexane or hexane-acetone extraction	GC/ECD	α-HCH β-HCH γ-HCH	NR	82–83% 73–77% 90–96%	Gupta et al. 1978

Table 6-1. Analytical Methods for Determining Hexachlorocyclohexane in Biological Materials *(continued)*

Sample matrix	Preparation method	Analytical method	Isomer	Sample detection limit	Percent recovery	Reference
Semen	Liquid-liquid extraction; cleanup with Florisil	GC/ECD GC/MS(NCI)	α-BHC β-BHC	0.02 ppb 0.32 ppb	72.5% 94.7%	Stachel et al. 1989
Semen	Extract with acetic acid; cleanup with Florisil; elute with petroleum-diethyl ether	GC/ECD	α-BHC β-BHC γ-BHC δ-BHC	NR	86.3% 101.3% 951.0% 101.6%	Waliszewski and Szymczynski 1983
Adipose tissue	Extract with organic solvents; reextract lipids on Florisil column; elute with hexane and concentrate	GC/MS	α-BHC β-BHC	5–50 ppb	>100% 80–100%	Liao et al. 1988
Adipose tissue	Extract fat from tissue with acetone-hexane; fractionate from fat by gel permeation chromatography with methylene chloride-cyclohexane; cleanup on Florisil column; inject	HRGC/ECD GC/MS	α-BHC γ-BHC β-BHC	1.2 ppb 1.4 ppb 3.0 ppb	>89% >88% >91%	LeBel and Williams 1986
Adipose tissue	Grind sample; isolate fat, extract residue in petroluem ether	GC/ECD	α-HCH β-HCH γ-HCH	10 ppb 20 ppb 20 ppb	NR NR NR	EPA 1980a
	Grind tissue; extract with acetonitrile and acetone; evaporate; extract with hexane	GC/ECD	β-HCH	80 ppb	98%	Barquet et al. 1981

Table 6-1. Analytical Methods for Determining Hexachlorocyclohexane in Biological Materials *(continued)*

Sample matrix	Preparation method	Analytical method	Isomer	Sample detection limit	Percent recovery	Reference
Milk	Solvent extract with ethylacetate-methanol-acetone; cleanup and concentrate using solid-phase extraction; elute with isooctane	HRGC/ECD	α-HCH β-HCH γ-HCH	0.5 ppb 1 ppb 0.5 ppb	83–105% 91–119% 80–96%	Prapamontol and Stevenson 1991
Milk	Homogenize sample; extract and cleanup using silica gel; elute with hexane/dichloromethane; concentrate; inject	HRGC/ECD	α-HCH β-HCH γ-HCH	0.002 ppm 0.009 ppm 0.004 ppm	125% 114% 125%	Butte and Fooken 1990
Brain	Homogenize sample in hexane; centrifuge; inject	GC/MS (NCI)	γ-HCH and metabolites	3 pg/L	NR	Artigas et al. 1988b

α-BHC = alpha-hexachlorocyclohexane; β-BHC = beta-hexachlorocyclohexane; γ-BHC = gamma-hexachlorocyclohexane; δ-BHC = delta-hexachlorocyclohexane; ECD = electron capture detection; GC = gas chromatography; α-HCH = alpha-hexachlorocyclohexane; β-HCH = beta-hexachlorocyclohexane; γ-HCH = gamma-hexachlorocyclohexane; δ-HCH = delta-hexachlorocyclohexane; HRGC = high-resolution gas chromatography; MS = mass spectrometry; NCI = negative chemical ionization; NR = not reported; TLC = thin-layer chromatography

by GC/MS is a reliable method for quantitation and identification of HCH isomers in semen (Stachel et al. 1989); sensitivity of GC/ECD is in the sub-ppb range with acceptable recoveries (Stachel et al. 1989). HRGC/ECD and GC/MS have also been used for detection and identification of HCH isomers in adipose tissue (LeBel and Williams 1986; Liao et al. 1988). During sample preparation, the use of gel permeation chromatography is effective for separation of the isomers from adipose tissue (LeBel and Williams 1986). This method is sensitive (low- to sub-ppb range) and has good recoveries (>88%) and precision (#0.12% RSD). Although sensitivity is not quite as good as that of GC/ECD, GC/MS is more specific. GC/MS is usually used as a confirmatory method, but it can be reliably used alone and produces excellent recoveries and good precision (Liao et al. 1988).

γ-HCH and its metabolites have also been detected in brain tissue using GC/MS in the chemical ionization mode (Artigas et al. 1988a). The use of GC/MS with negative ion chemical ionization (NICI) is preferred over electron impact mass spectrometry (EIMS) because the sensitivity using NICI is orders of magnitude better than with EIMS. GC/MS with NICI is also more selective than GC/MS with EI or GC/ECD (Artigas et al. 1988a). Another advantage of GC/MS with NICI is that identification and quantitation are performed without any purification or extraction procedures (Artigas et al. 1988a).

The phenolic metabolites of γ-HCH and the other HCH isomers have been measured in urine samples using GC/ECD (Angerer et al. 1981; Balikova et al. 1988). Sensitivity for this method is in the low-ppb range and recovery is excellent (95%); however, precision was not reported (Balikova et al. 1988). Thin layer chromatography (TLC) has also been used in conjunction with GC/ECD for identification of HCH isomers (Balikova et al. 1988). Although TLC does not achieve the same sensitivity (ppm range) as GC/ECD, sensitivity can be increased by extraction of a larger volume of urine. The combination of GC and TLC was reported to be a reliable confirmation tool for identifying compounds (Balikova et al. 1988). Angerer et al. (1981) developed a sensitive and specific gas chromatographic method for the simultaneous detection of 10 chlorinated phenols that appear in the urine of individuals exposed to γ-HCH. However, the study authors noted that both HCH and chlorobenzene compounds are commonly used as pesticides and that both are metabolized to chlorophenols. This suggests that detection of these metabolites does not distinguish between HCH, chlorobenzene, or pentachlorophenol (PCP) exposure. Edgerton et al. (1979) detected chlorinated phenol metabolites of HCH and PCP in the urine of experimental animals and exposed individuals by using GC/ECD. Discrimination between HCH and PCP exposure was possible through comparisons of metabolite profiles. However, detection of PCP in the urine may also be an indication of exposure to PCP or other compounds similar to HCH.

6.2 ENVIRONMENTAL SAMPLES

HCH residues are present in the environment because γ-HCH is used as an insecticide on a wide variety of vegetables, fruits, field crops, and on uncultivated land. The most commonly used methods for measuring HCH isomers in environmental samples is GC or HRGC combined with ECD or MS. Table 6-2 presents details on selected analytical methods.

HCH isomers have been measured in air using GC/ECD, HRGC/ECD, or GC with dual detection by ECD and electrolytic conductivity detection (ELCD) (Durell and Sauer 1990; Kurtz and Atlas 1990; NIOSH 1984; Stein et al. 1987; Zaranski et al. 1991). Polyurethane foam or Florisil adsorbent tubes are suitable for collecting air samples. The use of a simultaneous dual-column, dual-detector method (ECD and ELCD) was found to reduce the risk of false positive identifications without increasing the cost or time of analysis (Durell and Sauer 1990). Both columns were able to separate a large number of analytes with good reproducibility. Although ECD is more sensitive for halogenated compounds and has a lower detection limit (sub-ppb to low-ppm) than ELCD (low ppb), ELCD can greatly reduce matrix interferences. Precision and recovery were not reported for either detector (Durell and Sauer 1990; Kurtz and Atlas 1990).

The most commonly used methods for detecting HCH isomers in water (e.g., surface water, drinking water, sea water, groundwater, waste water, and rain) include GC or HRGC combined with ECD or MS (Allchin 1991; Barquet et al. 1981; Durell and Sauer 1990; EPA 1984, 1986a; Goosens et al. 1990; Kurtz and Atlas 1990; Lopez-Avila et al. 1989a, 1990b; Reding 1987; van der Hoff et al. 1991). To improve sample extraction and cleanup, the most current EPA method (Method 8120) used commercially available disposable Florisil cartridges instead of conventional Florisil cleanup (Lopez-Avila et al. 1989a). The disposable Florisil cartridges were simpler to use, shortened the analysis time, and reduced the overall cost of the analysis. The excellent precision, accuracy, and sensitivity (ppt range) of the results indicated that the revised method is reliable (Lopez-Avila et al. 1989a). Automated solid-phase extraction cartridges filled with silica and coupled on-line to GC/ECD have been effectively used to measure HCH isomers in water at low levels (ppt) (van der Hoff et al. 1991). This method is efficient and reproducible, with good recovery (>95%) and precision (<12% coefficient of variance (CV)) (van der Hoff et al. 1991). On-line liquid-liquid extraction coupled with HRGC/ECD is also a sensitive (ppb level) and reliable method (Goosens et al. 1990). A method validation study, conducted on EPA Method 508, for determining HCH isomers in finished drinking water using GC/ECD indicated the method was reliable, repeatable, and reproducible (Lopez-Avila et al. 1990b). Precision was good; recovery (>90%) was excellent. Sensitivity was in the ppb range (Lopez-Avila

Table 6-2. Analytical Methods for Determining Hexachlorocyclohexane in Environmental Samples

Sample matrix	Preparation method	Analytical method	Isomer	Sample detection limit	Percent recovery	Reference
Air	Collect air using filters and polyurethane foam; Soxhlet extraction; column cleanup and isolation; concentration; dual column detection	HRGC/ECD HRGC/ELCD		0.9 pg/μL 15.3 pg/μL	NR NR	Durell and Sauer 1990
Air	Collect sample in Florisil adsorbent tubes; elute with methylene chloride in pentane; concentrate in Kuderna-Danish evaporative concentrator; solvent exchange to hexane	HRGC/ECD		low pg/m^3	NR	Kurtz and Atlas 1990
Air	Trap in isooctane	GC/ECD		3 μg/sample	NR	NIOSH 1984 (Method 5502)
Air	Adsorb air sample on florisil; elute with 10% 2-propanol in hexane	GC/ECD	α-BHC β-BHC γ-BHC δ-BHC	0.25 pg/m^3	83% 88% 81% 87%	Stein et al. 1987
Surface water	Extract with hexane; concentrate; cleanup using automated solid-phase extraction technique	GC/ECD	α-HCH β-HCH γ-HCH δ-HCH	7 ppt 10 ppt 7 ppt 6 ppt	95.6% 98.2% 95.6% 95.9%	van der Hoff et al. 1991

Table 6-2. Analytical Methods for Determining Hexachlorocyclohexane in Environmental Samples *(continued)*

Sample matrix	Preparation method	Analytical method	Isomer	Sample detection limit	Percent recovery	Reference
Water	Extract twice with methylene chloride; dry with anhydrous sodium sulfate; concentrate; add hexane and concentrate by evaporation; cleanup on disposable Florisil cartridge and elute with hexane-acetone	GC/ECD	α-HCH β-HCH γ-HCH δ-HCH	11 ppt 31 ppt 23 ppt 20 ppt	96% 103% 96% 103%	Lopez-Avila et al. 1989a (Modified EPA Method 8120)
Drinking water	Extract with methylene chloride; solvent exchange to methyl ter-butyl ether; concentrate	GC/ECD	α-HCH β-HCH γ-HCH δ-HCH	0.025 ppb 0.010 ppb 0.010 ppb 0.015 ppb	94.6% 93.4% 94.2% 92.0%	Lopez-Avila et al. 1990b (EPA Method 508)
Drinking water	Stripping for water with an inert gas-helium	HRGC/ECD		0.003 ppb (Method 505); 0.006 ppb Method 508)	93–130%	Reding 1987 (EPA Methods 505, 508)
Drinking water	Separation with Na_2SO_4; extraction with CH_2Cl_2	GC/ECD	β-HCH	0.025 ppb	88%	Barquet et al. 1981
Water and waste water	Extraction with methylene chloride	GC/ECD	α-HCH β-HCH γ-HCH δ-HCH	0.003 ppb 0.006 ppb 0.004 ppb 0.009 ppb	NR NR NR NR	EPA 1984 (Method 608)

Table 6-2. Analytical Methods for Determining Hexachlorocyclohexane in Environmental Samples *(continued)*

Sample matrix	Preparation method	Analytical method	Isomer	Sample detection limit	Percent recovery	Reference
Water and waste water	Extraction with methylene chloride	GC/MS	β-HCH δ-HCH	4.2 ppb 3.1 ppb	NR NR	EPA 1984 (Method 625)
Water and waste water	Extraction with methylene chloride	GC/ECD	α-HCH β-HCH γ-HCH δ-HCH	0.003 ppb 0.006 ppb 0.004 ppb 0.009 ppb	NR NR NR NR	EPA 1986e (Method 8080)
Sea water	Extract twice with hexane; dry over anhydrous sodium sulfate; concentrate; cleanup using column chromatography with 5% deactivated alumina; concentrate	GC/ECD	α-HCH, γ-HCH	1 ppt	>85%	Allchin 1991
Ground-water	On-line liquid-liquid extraction of sample with isooctane and separation of aqueous and organic phases by a sandwich phase separator	HRGC/ECD	α-HCH δ-HCH	0.1 ppb	112% 119%	Goosens et al. 1990
Sea water, rain (lindane)	Liquid-liquid extraction; column cleanup and isolation; concentration	HRGC/ECD HRGC/ELCD		0.9 ppb 15.3 ppb	NR NR	Durrell and Sauer 1990

Table 6-2. Analytical Methods for Determining Hexachlorocyclohexane in Environmental Samples *(continued)*

Sample matrix	Preparation method	Analytical method	Isomer	Sample detection limit	Percent recovery	Reference
Sea water	Extract with methylene chloride; solvent exchange to hexane; cleanup on Florisil	HRGC/ECD	α-HCH, γ-HCH	low pg/L	NR	Kurtz and Atlas 1990
Soil	Extract with supercritical carbon dioxide or carbon dioxide modified with 10% methanol	GC/ECD GC/MS	α-BHC β-BHC γ-BHC δ-BHC	NR	77.43–93.6% 79.28–93.6% 80.63–121% 72.4–103%	Lopez-Avila et al. 1990
Soil	Dry sample with anhydrous sodium sulfate; extract twice with methylene chloride-acetone by sonication; filter; dry; concentrate; cleanup on disposable Florisil cartridge and elute with hexane-acetone	GC/ECD	α-HCH β-HCH γ-HCH δ-HCH	<40 ng/L	96% 103% 96% 103%	Lopez-Avila et al. 1989b (Modified EPA Method 8120)
Soil (lindane) (lindane)	Equilibrate with water; extract with acetone and hexane (1:1); wash with water and sodium chloride desiccate with anhydrous sodium sulfate; concentrate; add hexane; cleanup with SPE Florisil cartridge.	GC		5 ppm	108%	Noegrohati and Hammers 1992

Table 6-2. Analytical Methods for Determining Hexachlorocyclohexane in Environmental Samples *(continued)*

Sample matrix	Preparation method	Analytical method	Isomer	Sample detection limit	Percent recovery	Reference
Soil, sediment, waste sludge	Extract sample with methylene chloride-acetone by sonication; clean up using gel permeation chromatography processing of extracts dissolved in 1+1 butyl chloride-methylene chloride or 100% methylene chloride	HRGC/ECD, HRGC/MS	γ-BHC	NR	83–91%	Czuczwa and Alford-Stevens 1989
Soil	Hexane-acetone extraction	GC/ECD		NR	NR	AOAC 1984 (Method 29.013)
Soil	Extraction with methylene chloride followed by clean-up on Florisil column	GC/ECD, HSD	α-HCH β-HCH δ-HCH δ-HCH	3.0 ppm 6.0 ppm 4.0 ppm 9.0 ppm	NR NR NR NR	EPA 1986e (Method 8080)
Sediment	Extract using vapor phase distillation technique; dry isooctane extract; concentrate	GC/ECD	α-HCH γ-HCH	2.42 ppb 4.98 ppb	76% 40%	Schuphan et al. 1990
Milk	Selective extraction of HCH isomers on solid-matrix disposable column by means of acetonitrile-saturated light petroleum; concentrate; cleanup extract on Florisil minicolumn	GC/ECD	α-HCH γ-HCH β-HCH	NR	94% 105% 113%	DiMuccio et al. 1988

Table 6-2. Analytical Methods for Determining Hexachlorocyclohexane in Environmental Samples *(continued)*

Sample matrix	Preparation method	Analytical method	Isomer	Sample detection limit	Percent recovery	Reference
Milk	Extract fortified milk samples with acetone and n-hexane; centrifuge; evaporate organic phase; dissolve residues in ether	GC/ECD	α-HCH β-HCH γ-HCH δ-HCH	NR	95.7% 99.9% 83.4% 89.7%	Kapoor et al. 1981
Soil, water, wheat, rice, beans	Extract BHC from sample by activated charcoal; dechlorination of BHC to benzene; nitration of benzene to m-dinitro-benzene; reduction to m-phenylene diamine; diazotization and coupling to form azo dye	Spectrophoto-metry	γ-HCH	NR	≥89%	Raju and Gupta 1988
Mussels (lindane)	Extract with acetonitrile; separate from coextractives by liquid-liquid partition between acetonitrile and water/hexane; cleanup on Sep-Pak Florisil cartridge; elute in second eluate with 15% ethyl ether in hexane	GC/ECD		0.02 µg/kg	92–102%	Muino et al. 1991
Fish (lindane)	Extract residue using one-step matrix solid phase dispersion combined with Florisil column cleanup; inject into GC	GC/ECD		10 ng/g	82%	Long et al. 1991a

Table 6-2. Analytical Methods for Determining Hexachlorocyclohexane in Environmental Samples *(continued)*

Sample matrix	Preparation method	Analytical method	Isomer	Sample detection limit	Percent recovery	Reference
Fish	Petroleum ether extraction	GC/ECD		NR	NR	AOAC 1984 (Method 20.029)
Fish (lindane)	Combine with anhydrous Na_2SO_4; extract with petroleum ether/ethyl acetate; separate lipids with GPC; solvent exchange to iso-octane; add dry N_2 gas	GC/MS (NCI)		1.6 ppb	115%	Schmidt and Hesselberg 1992
Fruits and vegetables	Extract samples with acetonitrile; partition with sodium chloride saturated aqueous solution; concentrate	HRGC/MS	α-BHC β-BHC γ-BHC δ-BHC	0.05 µg/g (all isomers)	88% 93% 93% 112%	Liao et al. 1991
Vegetables (lindane)	Extract with methanol; and partition with sodium chloride and hexane; wash hexane layer with sodium chloride solution; desiccate with anhydrous sodium sulfate; concentrate; cleanup on SPE Sil-Florisil cartridge	GC		ppb range	87–137%	Noegrohati and Hammers 1992

Table 6-2. Analytical Methods for Determining Hexachlorocyclohexane in Environmental Samples *(continued)*

Sample matrix	Preparation method	Analytical method	Isomer	Sample detection limit	Percent recovery	Reference
Beef fat (lindane)	Extract residue using one-step matrix solid phase dispersion combined with Florisil column cleanup; inject into GC	GC/ECD		low ppb	85%	Long et al. 1991b
Animal fat and dairy products	For dairy products, extract fat with hexane; for animal fat, melt sample and remove fat; cleanup with gel permeation chromatography; further cleanup with Florisil if necessary; inject	GC/ECD	BHC	low to sub ppm	82%	Venant et al. 1989
Root vegetables and dairy products	Extract with CO_2 collect with *n*-hexane; evaporate; add *n*-hexane; load on Florisil column; elute with 1:1 (v/v) *n*- hexane/dichloromethane; evaporate; dissolve in *n*-hexane	GC/ECD	α-HCH γ-HCH	NR NR	10–100% 12–98%	Bernal et al. 1992
Beef	Extract with acetone-hexane; cleanup on Florisil column; inject	GC/ECD	β-BHC	sub ppm	78.1–88.3%	Tonogai et al. 1989

Table 6-2. Analytical Methods for Determining Hexachlorocyclohexane in Environmental Samples *(continued)*

Sample matrix	Preparation method	Analytical method	Isomer	Sample detection limit	Percent recovery	Reference
Tobacco	Soak in acetonitrile water mixture, extract with petroleum ether; shake with H_2SO_4	GC/ECD	α-HCH β-HCH γ-HCH δ-HCH	1.0 ppm 2.0 ppm 2.0 ppm 2.0 ppm	98.2% 92.9% 96.2% 88.2%	Waliszewksi and Szymczynski 1986
Wood (rasped)	Extract with toluene; sonify and centrifuge; inject	GC-MS		10 ppb	NR	Butte and Walker 1992

α-BHC = alpha-hexachlorocyclohexane; β-BHC = beta-hexachlorocyclohexane; γ-BHC = gamma-hexachlorocyclohexane; δ-BHC = delta-hexachlorocyclohexane; CH_2Cl_2 = methylene chloride; ECD = electron capture detection; ELCD = electrolytic conductivity detector; GC = gas chromatography; GPC = gas permeation chromatography; α-HCH = alpha-hexachlorocyclohexane; β-HCH = beta-hexachlorocyclohexane; γ-HCH = gamma-hexachlorocyclohexane; δ-HCH = delta-hexachlorocyclohexane; H_2SO_4 = sulfuric acid; HRGC = high-resolution gas chromatography; HSD = halogen specific detector; MS = mass spectrometry; Na_2SO_4 = sodium sulfate; NCI = negative chemical ionization; NR = not reported; SPE = solid phase extraction

et al. 1990b). The EPA-established analytical test procedures to analyze water, waste water, and drinking water samples use GC coupled with MS. EPA methods 608 and 625 are recommended to detect γ-HCH and other HCH isomers in surface water and municipal and industrial discharges (EPA 1984).

GC/ECD, HRGC/ECD, and HRGC/MS are the most commonly used methods to measure HCH isomers in soil, sediments, and solid wastes (AOAC 1984; Czuczwa and Alford-Stevens 1989; EPA 1986b; Lopez-Avila et al. 1989b, 1990a; Noegrohati and Hammers 1992b; Schuphan et al. 1990). More efficient extraction of the isomers from soil was obtained using a disposable Florisil cartridge (modified EPA Method 8120) prior to detection by GC/ECD (Lopez-Avila et al. 1989b). The method yielded excellent recoveries (>95%), and sensitivity was in the ppt range. Sample cleanup using a disposable solid phase extraction (SPE) cartridge with detection by GC yielded a higher recovery (108%) with excellent precision (4% CV). Although sample detection limits were not reported, sensitivity was in the ppm range (Noegrohati and Hammers 1992b). Sample cleanup using gel permeation chromatography and detection and identification by HRGC/ECD and HRGC/MS resulted in good recoveries (83–91%) and good precision (#5.1% relative standard deviation [RSD]) (Czuczwa and Alford-Stevens 1989); sensitivity was not reported (Czuczwa and Alford-Stevens 1989). A new technique, supercritical fluid extraction (SFE), has been applied to the analysis of soil samples (Lopez-Avila et al. 1990a). Recovery (>75%) and precision (<26% CV) are adequate. Because this is a relatively new method, the cost is higher than other accepted techniques. The vapor phase extraction technique has also been applied to the analysis of trace residues of HCH in sediments (Schuphan et al. 1990). The efficiency of this method was compared with conventional Soxhlet extraction and Florisil cleanup procedures. The results showed that recovery using the Soxhlet extraction method (73–81%) was better than with vapor-phase extraction (40–76%). The low recovery of γ-HCH (40%) was due to sample loss during concentration of the iso-octane extract (Schuphan et al. 1990); sensitivity was in the low-ppb range; precision was excellent (0.01–0.03% coefficient of variation).

GC/ECD and HRGC/ECD are the most commonly used methods for measuring HCH isomers in milk (DiMuccio et al. 1988; Kapoor et al. 1981), dairy products (Bernal et al. 1992; Venant et al. 1989), seafood (mussels and fish) (AOAC 1984; Long et al. 1991a; Muino et al. 1991; Schmidt and Hesselberg 1992), fruits and vegetables (Liao et al. 1991; Noegrohati and Hammers 1992), beef (Tonogai et al. 1989), and beef fat (Long et al. 1991b). Gel permeation chromatography is a suitable method for the cleanup of HCH residues in animal fats and dairy products (Venant et al. 1989); recoveries are good (82%). Although specific detection limits were not reported, sensitivity is in the low-to-sub-ppm range. Additional cleanup with Florisil is needed when residue levels are below 0.1 ppm; precision was not reported. High-pressure soxhlet extraction

coupled with Florisil column cleanup yielded recoveries up to 100% for α-HCH and γ-HCH in butter, if pressure, time, and sample volume in the extractor were optimized; detection limits and precision values were not reported. This method has also been used to detect γ-HCH residues in potatoes with similar recoveries (Bernal et al. 1992). A reliable and reproducible method has been developed to determine HCH residues in milk (DiMuccio et al. 1988). The procedure involves a single-step, selective extraction of residues from milk on a solid-matrix disposable column, clean-up with Florisil, and detection by GC/ECD. Although specific detection limits were not reported, sensitivity is in the low-ppb range. With this extraction procedure, the HCH residues are more readily extracted than milk lipids, and the addition of a small amount of acetonitrile to the milk significantly improved recoveries without increasing the amount of fat in the extracts (diMuccio et al. 1988). A reliable, rapid screening technique for extraction of residues from a complex biological matrix such as fat uses matrix solid-phase dispersion (MSPD) extraction, Florisil column cleanup, and detection by GC/ECD (Long et. al. 1991a, 1991b). This method has been used to measure HCH residues in beef fat and fish. Recovery (82–85%) is good; sensitivity is in the low-ppb range. The MSPD method overcomes many of the complications associated with traditional pesticide isolation techniques because it uses small sample volumes and involves few steps (Long et al. 1991a, 1991b). GC/MS with negative ion chemical ionization (NCI) with GPC cleanup is a rapid, accurate, and simple method to quantify γ-HCH in fish. Recoveries were excellent (115%) with good precision (8.9% RSD), and a detection limit of 1.6 ppb (Schmidt and Hesselberg 1992). An HRGC/MS screening method has been developed for the determination of pesticide residues in a variety of crop samples (fruits and vegetables) (Liao et al. 1991). This technique is a useful tool because it offers simultaneous detection and confirmation, which are not provided by ECD. This method, however, lacks the sensitivity achieved by ECD. Spectrophotometry has been used to measure HCH isomers in cereals (e.g., wheat, rice, and beans) with good recoveries ($89%) (Raju and Gupta 1988). This technique has also been used for other matrices such as soil and water (Raju and Gupta 1988). An accurate and simple extraction and cleanup method has been developed for capillary GC analysis of γ-HCH in vegetables. The sample was extracted with methanol and cleanup was executed on disposable SPE cartridges. Recoveries ranged from 87% to 137% (average 100%) with good precision (CV # 5%). Although no specific detection limits were reported, sensitivity is expected to be in the ppb range (Noegrohati and Hammers 1992).

HCH residues have also been detected in tobacco using GC/ECD (Waliszewski and Szymczynski 1986). Sensitivity is in the low-ppm range and recovery is excellent (88–98%) (Waliszewski and Szymczynski 1986).

GC/MS has been used to determine γ-HCH residues in wood preserving fluids on the surface of wood; the detection limit is 10 ppb. No recovery or precision values were reported (Butte and Walker 1992).

6.3 ADEQUACY OF THE DATABASE

Section 104(I)(5) of CERCLA, as amended, directs the Administrator of ATSDR (in consultation with the Administrator of EPA and agencies and programs of the Public Health Service) to assess whether adequate information on the health effects of hexachlorocyclohexane is available. Where adequate information is not available, ATSDR, in conjunction with the NTP, is required to assure the initiation of a program of research designed to determine the health effects (and techniques for developing methods to determine such health effects) of hexachlorocyclohexane.

The following categories of possible data needs have been identified by a joint team of scientists from ATSDR, NTP, and EPA. They are defined as substance-specific informational needs that if met would reduce the uncertainties of human health assessment. This definition should not be interpreted to mean that all data needs discussed in this section must be filled. In the future, the identified data needs will be evaluated and prioritized, and a substance-specific research agenda will be proposed.

6.3.1 Identification of Data Needs

Methods for Determining Biomarkers of Exposure and Effect. Methods are available for measuring HCH residues and/or their metabolites in blood serum (Barquet et al. 1981; Burse et al. 1990; Gupta et al. 1978; EPA 1980c; Saady and Poklis 1990), urine (Angerer et al. 1981; Balikova et al. 1988), semen (Stachel et al. 1989; Waliszewski and Szymczynski 1983), adipose tissue (EPA 1980c; Barquet et al. 1981; LeBel and Williams 1986; Liao et al. 1988), breastmilk (Butte and Fooken 1990; Prapamontol and Stevenson 1991), and brain tissue (Artigas et al. 1988a). However, examination of blood and urine is most frequently conducted to determine exposure because of the ease of sample collection with these media. The available methods are accurate and reliable for most of the media. However, sensitivity and precision data for measuring HCH residues in serum are needed. Although available methods can detect and quantify background levels of HCH in the population, there is no information to quantitatively correlate levels in these fluids with exposure levels. Additional quantitative information regarding the relationship between body and environmental levels of HCH might allow investigators to predict environmental exposure levels from measured body levels.

Methods are available to detect the chlorinated phenol metabolites present in the urine as a result of exposure to HCH (Angerer et al. 1981; Balikova et al. 1988). However, similar metabolites are detected following exposure to other pesticides. The identification of a specific urinary metabolite of HCH alone (e.g., chlorophenol) would not allow investigators to determine whether an individual has been exposed to HCH.

The individual isomers of HCH can be detected in serum, urine, adipose tissue, and semen of exposed individuals as indicated above in Section 2.7.1 Biomarkers of Exposure and Effect. Since no quantitative correlation has been made between body levels of HCH and adverse health effects based on existing data, we do not know if the methods are sensitive enough to measure levels at which biological effects occur. Further studies need to be undertaken to quantitatively correlate body levels resulting from HCH exposure and the occurrence of specific adverse health effects.

Methods for Determining Parent Compounds and Degradation Products in Environmental Media. Methods are available to detect HCH in air (Durell and Sauer 1990; Kurtz and Atlas 1990; NIOSH 1984; Stein et al. 1987; Zaranski et al. 1991), water (Allchin 1991; Barquet et al. 1981; Durell and Sauer 1990; EPA 1984, 1986a; Goosens et al. 1990; Kurtz and Atlas; Lopez-Avila et al. 1989a, 1990b; Reding 1987; van der Hoff et al. 1991), soil (AOAC 1984; Czuczwa and Alford-Stevens 1989; EPA 1986b; Lopez-Avila et al. 1989a, 1990b; Noegrohati and Hammers 1992; Schuphan et al. 1990), food (AOAC 1984; Bernal et al. 1992; Liao et al. 1991; Long et al. 1991a, 1991b; Muino et al. 1991; Noegrohati and Hammers 1992; Schmidt and Hesselberg 1992; Tonogai et al. 1989; Venant et al. 1989), milk (DiMuccio et al. 1988; Kapoor et al. 1981), tobacco (Waliszewski and Szymczynski 1986), and wood preserving fluid (Butte and Walker 1992). These methods are sensitive enough to measure background levels in environmental media. The precision, accuracy, reliability, and specificity of these methods are sufficiently documented. Research investigating the relationship between levels measured in air, water, soil, and food and observed health effects could increase our confidence in existing methods and/or indicate where improvements are needed.

6.3.2 Ongoing Studies

New methodology for improving multiple pesticide analyses of short-life residues in processed foods is being developed at the University of Tennessee, in Knoxville (L. Melton, investigator).